CHAPTER 4: DISCUSSION

This chapter aims to provide an objective interpretation of the statistical results, an explanation of the practical implications of the results and a discussion of how this investigation relates to other research in the same field. It will also discuss methodological issues, which might be improved on in the future, and suggestions for future research.

4:1 Results with reference to the hypotheses

This study investigated whether nutritional supplementation with a flavonoid antioxidant, could affect the symptoms of DOMS in an athletic population. The primary outcome of interest was whether there were any significant differences in DOMS response between the group using the flavonoid supplement (AO) and the control group using a placebo (P).

The statistical analysis did not show a significant difference between the groups in any of the three DOMS responses measured. i.e. no difference in perceived soreness, (Table 3), no difference in creatine kinase levels (Table 6), and no difference in peak torque scores for quadriceps muscles (Tables 9 & 11), or hamstring muscles. (Tables 13 & 15). The results of this study do not support the hypothesis that flavonoids reduce DOMS responses following eccentric exercise. The AO group recorded similar increases in perceived soreness (Figure 2) and creatine kinase levels (Figure 3), as the P group. Suprisingly, neither group showed a reduction in peak torque capacity (Figures 4,5,6 & 7), following the eccentric exercise, an anomaly that will be discussed later.
4:2 Results with reference to DOMS measures

The mixed analysis of variance test provided analysis of interaction effect between the AO group and the P group as described above, as well as analysis of difference between the repeated measures of each DOMS response that was measured.

Perceived Muscle Soreness

There was a significant difference in subjects' perceived soreness between the measures at different time points, with the exception of the measures at 24hrs and 48 hrs. (Table 4). Most important, was the large difference in perceived muscle soreness between the measure taken immediately after the eccentric exercise, and that taken 24hrs later. The mean increased from 1.645 to 3.642 on the VAS scale (p < .001), showing that physiological processes induced by the eccentric exercise had led to a delayed increase in muscle soreness, as had been intended. This confirms that the stepping exercise protocol had been effective in creating muscle damage, with an athletic population. The protocol, previously used by Gleeson (1998), had used recreationally active subjects, rather than an athletic group from a specific sport.

Some studies have found that muscle soreness continues to rise after 24hrs, peaking at 48hrs or 72hrs. (Connolly et al. 2003, Jeukendrup & Gleeson 2004). In this study, there was a levelling off at 48hrs, with no significant difference in soreness between 24hrs and 48 hrs in either group. This replicates findings of MacIntyre et al. (1996) and Gauche et al. (2006), who also tested runners. This may be due to the fact that the athletic sample were quicker to recover than a non-athletic sample, and/or due to the fact that the stepping protocol did not induce soreness to the same extent as other forms of muscle damaging exercise in the first place. Only 4 of the 14 subjects recorded a VAS score above 5 (pain with movement) at any time point. Subjects
experienced moderate soreness. Perception of pain varies between individuals and may depend on a number of variables including past experience, present mood, hormonal activity and immediate environmental circumstances. In this trial environmental factors were controlled, and past experience of the particular type of pain being recorded, would have had a level of consistency, since all subjects were runners. Most importantly, it was the change in level of perceived soreness that was of interest, rather than the level *per se*. Raw data (Appendix p77), show that the degree and timing of change in perceived soreness was quite consistent across all 14 subjects.

**Creatine Kinase Levels**

Creatine Kinase is an enzyme found normally within muscle and brain cells. Its function is to catalyse the conversion of creatine to phosphocreatine in an energy pathway. In healthy adults, measured levels of CK in the blood stream would be expected to fall between 25 – 200 u/l. (Jeukendrup & Gleeson 2004). However in conditions where muscle damage has been sustained, such as was induced by the stepping protocol in this study, muscle membrane damage leads to release of the enzyme into the blood stream, and a concurrent rise in assayed levels of CK. Hence its use as a biochemical marker of muscle damage in DOMS studies.

This is clearly demonstrated with one subject in the trial, (Subject 4 – recorded in raw data Appendix p77), who ‘inadvertently’ ran a 13 mile fell race the day before the trial, and recorded a baseline score of 1330 u/l CK. This subject’s CK data was excluded from the statistical analysis as a result, as it would have skewed the results disproportionally. This particular subject also provides a good example of the fact
that CK levels do not correlate well with performance and should not be used to predict performance. His peak torque output was one of the highest in the trial.

Changes in evidence of creatine kinase activity in the trial support the notion of delayed muscle damage occurring after the stepping protocol. Although there was a slight increase in mean creatine kinase level between the baseline level, and that tested immediately after the eccentric exercise, it was not significant (Table 8). However tests 24hrs later were significantly increased from a mean of 119.34 u/l at baseline, to 189.16 u/l at 24hrs. (p < .005) (Table 7). This is consistent with expectation, and with the concept of free radical activity causing further muscle membrane disruption in the period following initial muscle damage. As with perceived soreness, subjects in this study did not reflect the common trend of creatine kinase peaking at 48 hours. These subjects showed signs of recovery by 48hrs, as creatine kinase mean had dropped significantly to 148.02u/l, returning to the same level as that recorded immediately after the stepping protocol. It is likely that the recovery processes and endogenous anti-oxidant protection of this sample of runners, played a part in this phenomenon.

This pattern of CK activity was reflected in both the AO group and the P group, indicating that the presence of increased flavonoids in the plasma of the AO group, did not significantly reduce efflux of CK from muscle cells. However, as can be seen from Figure 3, 1 hour after the stepping trial the AO group had a mean CK level of 120 u/l only slightly higher than baseline, while the P group had risen to a mean of 175 u/l. While this was not statistically significant, it may indicate an effect from the supplement in a period extending to approximately 4 hours post supplementation. By
24 hours, the mean CK level of the two groups was virtually identical. This may suggest that flavonoid levels of the AO group had dropped to normal range in the interim period.

**Peak Torque Measures**

The third measure of DOMS was a measure of performance, testing for peak torque of quadriceps and hamstring muscles at two speeds on a Biodex dynanometer. Warren et al. (1999) define muscle function as the ability to exert force under a given set of conditions, in this case, those set by the Biodex protocol. Warren et al take the view that measures of muscle function provide the best means of evaluating the magnitude and time course of muscle injuries resulting from eccentric contractions. The expectation of the investigator was that peak torque scores would be reduced following the DOMS induced by eccentric exercise, and that potentially, the AO group would show less reduction in performance and quicker recovery to base line scores that the P group. Given the outcome of the measures already described, and the fact that muscle soreness and creatine kinase were most evident at 24 hrs, it would be anticipated that peak torque performance would be affected and scores reduced at this time. However this was not the case. In fact peak torque scores did not change significantly between any of the repeated tests. (Tables 10,12,14 & 16)

Evaluated in isolation, these results appear to suggest that no muscle injury actually occurred, and there was no measurable magnitude or timescale of injury detectable. Fortunately the other dependent variables contradict this interpretation. A possible explanation for these peak torque results is provided in section 4:4
It has been suggested that Type II muscle fibres are preferentially recruited during eccentric exercise, and that consequently these fibres would sustain more damage during a bout of eccentric exercise (Gullick et al. 1996). It has also been proposed that fast-twitch units are better able to exert a given force at a shorter length than other fibres and consequently the stretch resulting from eccentric contractions leads to greater disruption of type II fibres (Byrne; Twist & Eston 2004). If this was the case, and the isokinetic dynanometer measurements were sufficiently sensitive, then it might be expected that peak torque at the faster velocity, (in this study 240 seconds), (Figures 5 & 7) would be reduced more than that at the slower speed. (60 seconds). (Figures 4& 6). This was not evident in either the AO group or the P group in these results, with muscle performance at both velocities showing no significant change, nor a significant difference between speeds. Thus with this sample of runners, there has not been evidence of a variation in muscle damaging effect from eccentric exercise, to fast and slow twitch muscle fibres.

In summary, neither the ingestion of a flavonoid supplement, nor (apparently), a prior bout of eccentric exercise, made a difference to the subjects’ peak torque capacity.

4.3 Results with reference to related research
Since the success of past research in using nutritional supplements to reduce the symptoms of DOMS varies to a great extent, it is impossible to state that this study either supports or refutes current views. In addition, it is likely to be the first study to investigate flavonoids as a single, isolated supplement in this way. What can be stated, is that this investigation has not shown flavonoids, used in isolation and in a
manufactured, supplement form, to be an effective means of altering the course of DOMS. Further, it can be assumed that this form and dosage of flavonoid has not attenuated free radical activity after exercise–induced muscle damage and acute inflammation.

The results of this study, are consistent with those of Thompson et al. (2003), the design of which was used as a model in many respects, by this study. Thompson et al found no reduction in DOMS in the trial group who supplemented with 400mg Vitamin C for 3 days post exercise, which led to their conclusion that: “Vitamin C alone, consumed wholly after exercise, is unable to deliver antioxidant effect to the muscles with sufficient expediency to improve recovery.” There is an implication that whilst not effective alone, vitamin C taken together with another or some other antioxidants, may still be effective. Much the same can be concluded for the flavonoids used in this study. Bloomer et al. (2004), using a combination of 1000mg Vitamin C, 268mg Vit E and 90 µg selenium per day, taken 14 days before and 3 days after the trial, did find a reduction in soreness and CK, but no difference in performance. This could be due to the fact that the combination produces anti-oxidant activity both in plasma (Vit C & selenium) and in lipid membranes (Vit E). Like Vit C, flavonoids are water soluble, and thus may be more effective in combination with other nutrients.

Gauche et al. (2006) used a vitamin and mineral complex known as ‘Isoxan Endurance’, which contained 200mg Vitamin C; 32mg Vit E; betacarotene and selenium along with some Vitamin B complexes. The subjects supplemented for 21 days before and 2 days after the trial. This produced a quicker return of performance by 24 hours, (measured by Maximum Voluntary Contraction) in the treatment group. CK and perceived soreness were not recorded.
Subjects in the Thompson et al. (2003) study received 200mg of Vitamin C immediately after the exercise protocol, and then twice a day for the next three days, in accordance with best strategy for bioavailability. The flavonoid in this study, was only administered once a day, in accordance with manufacturer’s instructions. However it is very likely that an additional dose would have maintained plasma levels of flavonoids in the AO group more efficiently, as like Vitamin C, flavonoids are water soluble and chemically unstable.

It may be deduced from the combination of results from this study and previous research, that:

i) a combination of anti-oxidants is more effective that a single source of anti-oxidant.

ii) Supplementation before muscle damaging exercise as well as after the exercise, is more effective than only after the exercise.

iii) It is likely that Vitamin E is required for approximately two weeks prior to the exercise, and that Vitamin C, flavonoids and any other water soluble compounds, are likely to be best administered a few hours before and for 2-3 days after the exercise.

4:4 Methodological limitations

Peak Torque results

In this study, subjects were given one practice session on the Biodex dynamometer. They performed the protocol (Appendix p 64), using their right leg, in order to gain
familiarity with the test requirements and cues. This was done immediately prior to proceeding with the baseline measurement, using the left leg.

It is possible that this did not provide enough habituation for the subjects to perform to their potential on the first baseline test, leading to scores for the baseline, that were lower than they should have been. This would account for the apparent lack of change in peak torque output over the following three tests. e.g. subject \( a \) scored 232 for quad peak torque at baseline, with subsequent scores of 237; 232; 229. If however the baseline score was depressed due to ineffective habituation, the baseline score might have been up to 10 points higher, at 242. The subsequent score would then reflect a reduction in peak torque output. Gleeson and Mercer (1996) note that the process of learning a movement on the dynamometer includes a phase of accommodation, in which specific movements and neuro-muscular patterns are acquired. This may not have transferred from the right leg to the left leg successfully in this study. Gleeson and Mercer also indicate that a number of replications of the test may be required to produce sufficient sensitivity. Thus in future, subjects may be required to either be habituated on a day prior to the start of the trial, or at least 3 hours before the trial, in order to recover. The difficulty is balancing reducing motivation from performing multiple test repetitions, with eliciting a reliable baseline measurement.

**Bioavailability of flavonoids**

A number of factors were considered and weighed against each other when selecting a source of flavonoid for use in this study. A manufactured supplement was selected, firstly because it provided a consistent, measurable quantity of the flavonoids to be
administered, and it contained a wide spectrum of flavonoids that would have been entirely impractical for subjects to have eaten from food sources. However it is thought that absorption rate, potency and chemical stability is not the same in this form, as it would be for flavonoids consumed in whole fruits and vegetables, partly due to the fact that many flavonoids are bound to sugars as beta-glycosides, which affects their passage through the gut wall. (Hollman & Katan 1997). This is a factor, which reduces the ability of the study to quantify the level of flavonoid absorption in the trial group.

Due to financial and time constraints, there was no prior measurement made, of plasma anti-oxidant capacity, following ingestion of the flavonoid supplement. A pilot of this nature would have confirmed that ingestion of the Bioflavone 1000 supplement, actually did result in a rise in anti-oxidant capacity. This can be done using the Frap assay procedure (Benzie & Strain 1996). Future studies could make use of this analysis, rather than having to assume that the supplement was absorbed successfully. It could also be used to assess how long after ingestion, peak anti-

oxidant capacity is reached, and when it returns to baseline.

If resources are available, there is also a means of directly measuring oxidant injury in vitro, as opposed to using indirect measures such as CK or malondialdehyde. This is by measurement of F2 isoprostanes, which according to Prior (2004) has emerged as the most reliable approach to assessing Oxidative Stress (OS) in vivo. F2 isoprostanes are a series of prostaglandin-like compounds formed from free radical initiated peroxidation of arachidonic acid (Prior 2004). Use of this analysis would allow investigators to track the pattern of OS in DOMS.
A second issue was the timing of supplement administration. Since the manufacturer recommended just one tablet per day, subjects took the capsule once every 24 hours. This may have meant that plasma levels of the flavonoids dropped progressively and were not maintained above those of the P group for the full 24 hours, as was intended. With hindsight, it may have been better to have split the tablet in to two doses, to be taken every 12 hours.

Although not statistically significant, the creatine kinase response of the AO group at test 2, i.e 1 hour after the eccentric exercise, was only marginally elevated from baseline, compared with the P group. This can be seen clearly in Figure 2. This was three hours after the administration of the flavonoid, and may suggest that there was some protective effect for a short period, while flavonoid levels were potentially high in the AO group. This effect was then lost 24 hours later, when CK was at a peak for both groups. A similar trend is evident in the peak torque results for quads and hamstrings at 60 seconds. (Figures 3 & 5), where there is a clear difference in performance between the groups at test 2, where the AO group performs better than the P group. This may suggest that the anti-oxidant capacity of the AO group was raised for a short period, in the region of 3-5 hours post ingestion, and that this effect did have some impact on DOMS response at this time. With a subsequent drop in flavonoid level and anti-oxidant capacity, no further benefits were evident.

This may support the need for more regular ingestion of flavonoids during the 24 hour period post muscle damaging exercise, when free radicals are active. There is scope for further investigation along these lines.
4.5 Conclusion

The potential of antioxidant nutrition to protect against and reduce the deleterious effects of free radical activity in muscle cells following intense or unaccustomed exercise is a relatively recent area of investigation. As findings from the more rigorous studies are brought together and positive results are linked together in the pursuit of a common goal, it is possible that more effective and more predictable interventions with anti-oxidant nutrients will develop. The interaction between the human endogenous anti-oxidant system, and exogenous anti-oxidant nutrition needs to be better understood, as does the process of adaptation during specific training, that provides athletes intrinsic resistance to DOMS in the adapted muscle groups.

This study has not supported the use of flavonoids alone to reduce DOMS and the hypothesis of the study has therefore been rejected. However the early responses of the AO group, i.e. within the first hour post the trial, warrant further investigation. With the suggested improvements to experimental procedures, there is an opportunity for further progress in researching the use of anti-oxidant nutrition in sports applications.

4.6 Future Research

- A key extension of this investigation, would be a follow up that tests a combination of Vitamin E and flavonoid supplementation. With Vitamin E supplemented for 14 days before a trial and for 2 days after, and flavonoids supplemented for approximately 3 hours before and 2 days after, the possibility of finding an effective reduction of DOMS seems more likely.
• It would be helpful to have more controlled trials on the absorption pattern of flavonoids. e.g. a trial comparing absorption of a manufactured flavonoid supplement, with a comparable food-based source and a trial following the absorption curve of a flavonoid supplement. The latter would assist with planning timing and spacing of supplement administration.

• Testing of flavonoid supplementation in a medical setting, for use with soft tissue inflammatory conditions is a real possibility, with great health benefits if found to be effective. Conditions such as tendonitis could be used in a trial, comparing the effects of flavonoids or combined anti-oxidants, with NSAIDs. Many people cannot be prescribed NSAIDs because of dangerous side effects and costs of NSAID prescriptions reached £247 million in UK in 2004. (Barton, Avery & Whynes 2006).